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Chemical stimulation of a green alga*

BURTON EDWARD LIVINGSTON

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INTRODUCTORY

That certain activities of plants, notably that of growth, are accelerated by many mineral poisons when the latter are applied in very great dilution has long been known in a general way. The nature of this toxic stimulation and how it is brought about are, however, as little known as are the vital functions themselves. Indeed, until very recently no quantitative data have been available regarding such responses, either in plants or animals. Thus it appeared worth while to undertake a comparative study of toxic stimulation, using a single form of plant and as large a number of poisons as possible. The present paper embodies the results of

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* The principal part of the investigations described in this paper was carried on at the New York Botanical Garden by the aid of a research scholarship from that institution during September to December, 1903.

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the first section of this problem, and deals with the effects of what are termed the positively charged ions from dissociated mineral salts.

In the following pages the term *stimulation* will be used to denote any catalytic effect upon vital activity, brought about by any change of the conditions under which the organism is living. Thus, any substance which, upon entering the protoplasm, causes either an acceleration or a retardation of certain functions, is a stimulating agent. Death itself, when it results from poisons at least, is merely the last of a series of stimulation responses, or, rather, it is the final summation of such responses, and appears as a single one only because we have not yet been able to unravel the vast tangle of activities of which it is the resultant.

Two ends must be held in view in a research of this kind. First, analysis is to be made of the conditions which bring about the response, and determination of their manner of action. Second, knowing in this way something of the sets of conditions which stimulate, it will eventually be possible, it is hoped, to interpret the latter so as to throw light upon the nature of the stimulated processes themselves. It is by this means that we may hope to gain more definite knowledge of the complex system of energy changes which make up vitality. Thus the problem may be approached in somewhat the same manner as that in which the chemist attacks a new organic compound, by studying in a quantitative way the effects of various reagents upon it. In this case of the chemist, the reagents are at least better known than the body to which they are applied, and the same must be true in physiological work. For a study of toxic stimulation, a knowledge of reagents comes, of course, from the realm of physics and chemistry.

The organism used in the experiments here described is the same form of *Stigeoclonium* whose responses to changes in external osmotic pressure were worked out some time ago.* A brief

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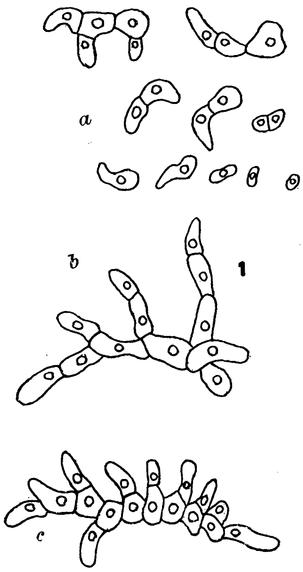
* Livingston, B. E. On the nature of the stimulus which causes the change of form in polymorphic green algae. Bot. Gaz. 30 : 289–317. 1900. — Further notes on the physiology of polymorphism in green algae. Bot. Gaz. 32 : 292–302. 1901. — The rôle of diffussion and osmotic pressure in plants 132–137. Chicago. 1903. (These pages are included in the reprint of the last chapter of the volume, entitled, The influence of the osmotic pressure of the surrounding medium upon organisms. Chicago. 1903.) A portion of the résumé here given is taken from the last reference.

résumé of the previous results is here given. In nutrient media whose osmotic pressure is from 323.7 cm. to 647.4 cm. of mercury, the alga appears as groups of spherical cells with thickened and somewhat gelatinous walls. Multiplication takes place rather slowly, cell-division occurring in all directions, and the daughter cells immediately become spherical, so far as this is not prevented by adjacent cells. Often this process of rounding off results in the entire separation of cells, so that a culture of this sort usually exhibits numerous free cells of perfectly spherical form. In weaker solutions, whose pressure is below 161.8 cm. of mercury, the daughter cells elongate into branching filaments composed of cylindrical cells and having the typical appearance of the smaller forms of the genus *Stigeoclonium*. Growth is more rapid here than in the strong solutions. If filaments are transferred to a strong solution their cells round up and often separate, thus producing the other form, and growth continues in the manner first described. In the weak solutions zoöspores are formed in great numbers and germinate to form filaments ; in the strong solutions they are not formed at all, and if transferred to such media they fail to germinate, many of them, however, passing by direct enlargement to the conditions of free spherical cells which later behave in accordance with the pressure of the medium, as outlined above. The biciliate zoöspores are produced by simple segmentation of the entire cell-contents, which are freed by a final bursting of the sporangium wall.

A resting zoöspore and several stages in the germination of these bodies are shown at *a*, *fig. 1* ; at *b* is shown a more mature filament, as these normally occur floating on the surface of the weak solution ; while at *c* is shown a form with more crowded branches, which is usually exhibited on the bottom of the culture dish, where air has not such free access. The spherical form, known usually in such algae as the palmella form, is shown in *fig. 2*. The groups of cells at *a* and *c* have developed from filaments which were transferred from a weak to a strong solution. The filamentous form is still to be seen in both groups, although it has well nigh disappeared in the lower. At *b* are some free cells in process of division which would result in groups of the palmella form.

This investigation was begun and about half finished in the laboratories of the New York Botanical Garden, where it was my good fortune to hold a research scholarship during the autumn of 1903. Through the kindness of the Director in Chief and of the Director of the Laboratories, the facilities for the work were practically unlimited. The experiments were completed at the Hull Botanical Laboratory of the University of Chicago during the winter and spring just passed.



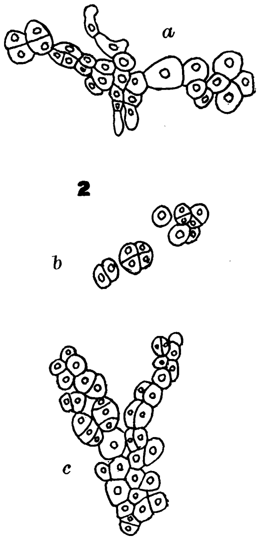


FIGURE 1. Normal filaments, from weak nutrient solution.
FIGURE 2. Normal palmella, from strong nutrient solution.

METHODS

A modification of the well-known Knop's nutrient solution was used as a basis for the culture media and for the controls. The differences between this and the true Knop's solution are such as to make the former more easy of preparation when the chemistry of the mixture is to be considered. The formula, and certain physical properties of this standard culture medium are shown in the following table. The concentration data for the salts are given in terms of a normal solution, *i. e.*, one gram equivalent per liter of solution.* The osmotic pressure data are computed from Δ_f, the lowering of the freezing point below that of distilled water †, measured in Centigrade degrees.

FORMULA AND OSMOTIC PRESSURE OF NUTRIENT MEDIUM

Salts	Concentration.	Lowering of freezing pt. (Δ_f)	Osmotic pressure.		
			mm. Hg.	Atmospheres	M.
$Ca(NO_3)_2$	15*n*/10,000	0.006°C.	60.09	0.079	0.0035
KNO_3	4*n*/10,000				
$MgSO_4$	4*n*/10,000				
K_2HPO_4	4*n*/10,000				
$Fe(NO_3)_2$	*n*/100,000				

The above solution was made up from normal solutions of the component salts. Practically no $Ca_3(PO_4)_2$ is precipitated if the K_2HPO_4 is added to the other salts only after the addition of nearly all the required water.

In the control medium just described the alga takes the filamentous form, and zoöspores are plentiful. For the palmella form a solution of one hundred times the strength of this was used. The iron salt, however, was not increased in amount.

On account of the extreme complexity of the general question of toxic stimulation, it seemed expedient to work with but one of the two forms of this alga. By the use of poisons it has been so far impossible to cause a strong solution to produce other than the palmella form, but weak solutions can be made to produce this form at will, as will be shown in this paper. Therefore the filamentous form was chosen as the most responsive and thus the best suited to the work in hand.

On account of the well-known toxicity of certain metals, and in order to simplify the otherwise almost hopelessly complicated methods, the problem was further restricted by confining attention to the cations * alone. In order to do this it was necessary to use as stimulating agents salts whose anions were already present in the control solution described above. Soluble nitrates and sulfates were chosen for this purpose. Normal or decinormal solutions of these salts were made up as accurately as possible, and these were diluted to the concentrations needed for the cultures. Since the addition of the poison to the nutrient solution would necessarily dilute both solutions to some degree, the following method of mutual dilution was adopted. The nutrient solution was made up to nine-tenths of its required volume, while the poison solution for each culture was prepared ten times its required concentration, or one tenth of its required volume. Thus, for control culture, 9 c.c. of stock nutrient solution plus 1 c.c. of water was used, and for any concentration of poison (say kn, k being any fraction of normal), the culture medium was composed of 9 c.c. of nutrient solution plus 1 c.c. of 10 kn poison. An example will illustrate this: Suppose that it was desired to test the effect of $n/10,000$ H_2SO_4. 1.5 c.c. of $n/1$ $Ca(NO_3)_2$ was taken, together with 0.4 c.c. each of $n/1$ KNO_3 and $n/1$ $MgSO_4$, and 1.0 c.c. of $n/100$ $Fe(NO_3)_2$. After mixing, these were diluted to a volume of 899.6 c.c. and then there was added 0.4 c.c. of $n/1$ K_2HPO_4. A further addition of 100.0 c.c. of water would have produced the control medium. To 9.0 c.c. of this "9/10 dilution," was added 1.0 c.c. of $n/1000$ H_2SO_4. It is readily seen that through mutual dilution both sets of ions come to be of the desired concentration, the H_2SO_4 being now $n/10,000$. The slight increase in the osmotic pressure of the experiment solution over that of the control, due to the addition of the poison itself, is, in most cases, physically negligible (because of the extreme dilutions used), and is always physiologically so (because of the comparatively high concentration limits for the response of this form, as previously † determined). Poisons of as high concentration as $n/100$ have been used in only a few cases.

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* Throughout this paper the decimal system will be used to denote fractions of normal solutions rather than the cumbrous method of dilutions by one half commonly in use by chemists. One is easily reducible to the other, but it seems that for modern workers the decimal system is by far the better. The form of the common fraction is retained on account of a somewhat greater ease of reading.

† For the method of making this calculation, see Livingston, B. E. The rôle of diffusion and osmotic pressure in plants, 37 (Chicago, 1903), and the references given on that page and at the beginning of the volume. The osmotic pressure, in millimeters of mercury, of a solution at 25° C. (P_{25}) is approximately given by the formula : $P_{25}=10{,}014.84\ \Delta_f$. This quantity in terms of M (22.3 atmospheres, the calculated pressure of a molecular solution of a non-electrolytic and non-hydrating body) is given by the formula : $P_{25}=0.59\ \Delta_f$.

The poison salts added to the nutrient solutions were also

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*The terminology of the ionic theory is here used, merely because it is most convenient.

†Livingston, B. E. 1900 and 1901, *loc. cit.*

used in such dilution that the slight increase in NO_3 and SO_4 ions caused by the addition could have no effect upon the plant. To test this, variations in the amount of nitrate and sulfate were tried over a greater range than that required for the main series of experiments, but without any response in the plant. This is in agreement with the results published previously (*loc. cit.*, 1900), wherein the effect of a decrease of nine-tenths in the amount of any salt in Knop's solution and an increase in the other three main salts sufficient to keep the pressure constant, was found to be without response in the plant.

Kahlbaum salts were used throughout the work and the utmost pains were taken to have the concentrations correct. All water was distilled in block tin and redistilled in glass.

Pure cultures of the alga were made in the standard nutrient solution and from these the inoculations were make for the experiments. These inoculations were made with wood tooth-picks in place of the usual needle. Sterilization was found not to be necessary, there being no organic bodies in the solutions, and a new tooth-pick was used for each individual culture. Upwards of fifty cultures were usually made at a time, the poison solutions having been prepared in small bottles a day or two before and kept stoppered. The dishes used were the Bausch & Lomb glass culture dishes, about 4.5 cm. in diameter and 2.0 cm. high, with a lid fitting down upon a shoulder after the manner of a pill-box. Ten cubic centimeters of solution were used for each culture. At the New York Botanical Garden the experiments were carried on partly on glass shelves against the pane of an east window, partly in a cool experiment house, and partly on tables directly under a large skylight. At the Hull Laboratory they were placed on glass shelves against a west window. The alga does not require strong light and grows best at a comparatively low temperature. Thus an east or west window of a ordinary laboratory room is well suited for its growth. Cultures came to maturity somewhat earlier in the experiment house than in the laboratory. An experiment ran from twelve to twenty days, being examined *in the dish*, with the low powers of the microscope at critical times, — every day or two for the first few series.

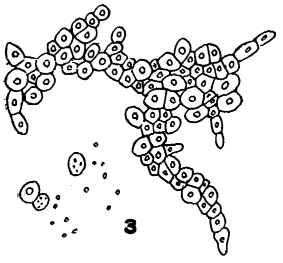
All of the data given are derived from cultures repeated several times. The number of the cultures amount to 1,048 in all.

EXPERIMENTATION

I. PRELIMINARY. — The responses of the filamentous form of this plant to toxic salts are of three somewhat distinct types: (1) death, (2) change in phenomena of growth, and (3) change in phenomena of reproduction. The fatal concentration was determined for most of the salts tested. At a concentration somewhat below the fatal strength, all of the salts cause the filaments to take the palmella form. That is, the presence of the toxic salt causes the plant to behave in this regard as though it had been placed in a solution of relatively high osmotic pressure. The cylindrical cells become spherical and division proceeds in the normal manner as described for the palmella form. But many of the salts also produce, at a certain concentration, another response, namely an acceleration in the production of zoöspores, so that a poisoned culture shows zoöspores sooner and in greater number than does the control without the poison. This often occurs at a concentration where only the palmella form is produced, and here the zoöspores cannot germinate as filaments. They act in this case as though they had been placed in a strong solution. Some of them die and others simply enlarge and become free palmella cells. These points will be discussed more fully farther on.

II. STATEMENT OF RESULTS. — Following is a description of the responses to the different reagents employed. The salts are arranged in alphabetical order according to the English name of the metal. The figures are all camera drawings and the magnification is about 300 diameters.

1. Aluminium nitrate ($Al_2(NO_3)_6$). — Filaments are all killed in *n*/10,000. In 5*n*/100,000 most of the cells die, but what few resist the poison for the first week develop into the palmella form. No zoöspores are produced. (See *fig. 4*, wherein are shown a parenchyma-like mass of cells, three groups of two cells each, and two single cells. Of one of the groups of two, one cell has died after making the palmella form, as is indicated by the granules within. The similar granules lying about the culture are remains of dead cells.) In a concentration of *n*/100,000 the majority of the cells become spherical or nearly so but a number still die. Also, there is here a marked production of zoöspores, which form spherical cells immediately after germination, usually by the time they have reached the two-celled stage. (See *fig. 5*. Here the dead filament-cells are denoted by dotted contents, the same methods will be used in the following figures. A number of empty sporangia are shown at *a*, together with zoöspores in various stages.) In weaker concentrations the filaments persist, zoöspore acceleration being still evident in $5n/1{,}000{,}000$. In $n/1{,}000{,}000$ the culture is normal for a weak solution. Here zoöspores are produced but not as soon nor in such great number as in the stimulated cultures.



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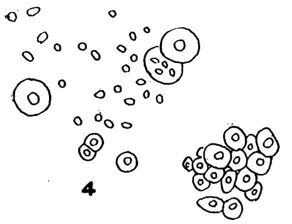

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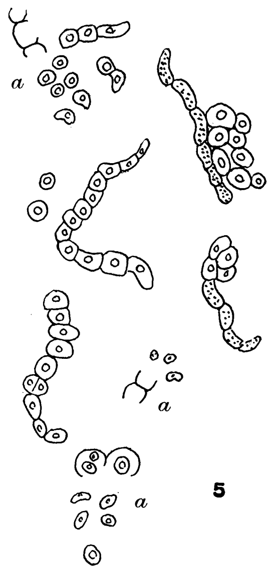

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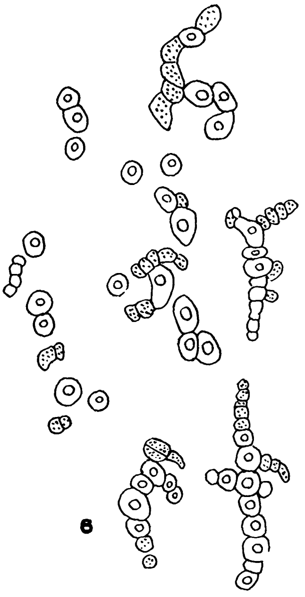
FIGURE 3. Palmella and dead cells from $5n/100{,}000$ $Al_2(SO_4)_3$.
FIGURE 4. Palmella and dead cells from $5n/100{,}000$ $Al_2(NO_3)_6$.
FIGURE 5. Palmella and zoöspores from $n/100{,}000$ $Al_2(NO_3)_6$.

2. *Aluminium sulfate* ($Al_2(SO_4)_3$). — This salt acts in the same way as does the nitrate, and at the same concentrations. (See *fig. 3*, showing a large group of palmella still exhibiting traces of

the filamentous form from which it came, and several dead cells with granular remains of others.)

3. Ammonium nitrate (NH_4NO_3). — The killing strength was not determined ; it lies above *n*/100. In the last named concentration a large proportion of the cells die, the remainder becoming palmella. The same is true of *n*/1,000 but more cells live. (See *fig. 6*, showing palmella cells arising from cylindrical filamentous ones, by the survival of the most resistant, apparently.) In 5*n*/10,000 and 6*n*/10,000, the palmella form is still more or less apparent, though good filaments usually persist. There is in the last named strengths an acceleration of zoöspore production, as in the aluminium salts. At *n*/10,000 all traces of palmella form are lost and the zoöspore activity has sunk to normal, *i. e.*, this strength gives the usual growth for a solution of low osmotic pressure.



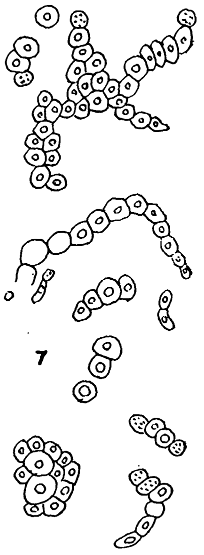


FIGURE 6. Palmella and dead cells from *n*/1,000 NH_4NO_3.

FIGURE 7. Palmella, traces of filaments, sporangia, and zoöspores, from 5*n*/10,000 $(NH_4)_2SO_4$.

4. Ammonium sulfate ($(NH_4)_2SO_4$). — The results are the same as for the nitrate. (*Fig. 7* shows palmella, several fairly typical filamentous cells, and sporangia.)

5. Barium nitrate ($Ba(NO_3)_2$). — With this salt, *n*/100 kills practically all cells, although sometimes a few of the palmella form will survive. The palmella form is typically produced in 5*n*/1,000, and more slowly in *n*/1,000. In 5*n*/1,000 to some extent, but especially in *n*/1,000, there is again an acceleration of zoöspore production, which activity falls back to normal at a concentration of 5*n*/10,000.

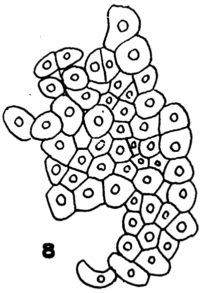
6. Calcium nitrate ($Ca(NO_3)_2$). — This salt is a component of the nutrient solution itself and therefore it is difficult to determine its effect upon the plant without having recourse to other methods than the ones here used. The experiments indicate that it produces palmella at 5*n*/100, and that there is a zoöspore acceleration from *n*/100 to 5*n*/1,000. The palmella response is perhaps partly due to osmotic pressure.

7. Cadmium nitrate ($Cd(NO_3)_2$). — The lowest dilution producing death to all cells is *n*/10,000, usually 5*n*/100,000 kills the majority of the cells. The typical palmella form is produced in concentrations of from 5*n*/100,000 to *n*/100,000, and zoöspore acceleration appears in the latter concentration and continues in weaker ones till the cultures become normal in the vicinity of 5*n*/1,000,000. It is often difficult to tell where acceleration of zoöspore production actually ceases ; the markedly increased activity of the higher concentrations grades almost imperceptibly into the normal production of these bodies in the weaker ones.

8. Cobalt nitrate ($Co(NO_3)_2$). — The killing strength is from *n*/1,000 to 5*n*/10,000. The palmella form is produced in 5*n*/10,000 to *n*/100,000, and zoöspore stimulation at *n*/10,000 to 5*n*/1,000,000, below which concentration the cultures are normal. (*Fig. 8* shows palmella from *n*/10,000 concentration.)

9. Cobalt sulfate ($CoSO_4$). — The responses here follow accurately those with the nitrate. (*Fig. 9* shows palmella in 5*n*/1,000,000, while *fig. 10* shows nearly normal filaments in one-fifth of that strength.)

10. Copper nitrate ($Cu(NO_3)_2$). — The killing strength is from *n*/100,000 to 5*n*/1,000,000. In the latter strength sometimes a few palmella cells remain. Filaments are inhibited, thus giving the palmella form, in strengths from 5*n*/1,000,000 to 5*n*/10,000,000. The palmella form in solutions of this salt is usually somewhat bluish green in color. There is an acceleration of zoöspore production at *n*/1,000,000. At *n*/10,000,000 the culture is normal.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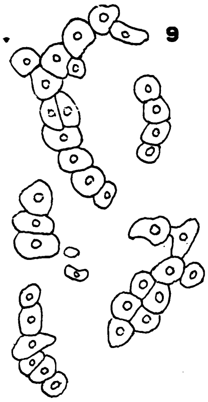
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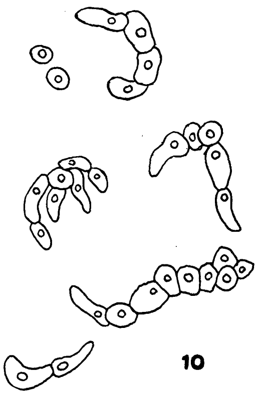
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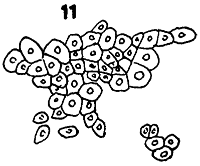
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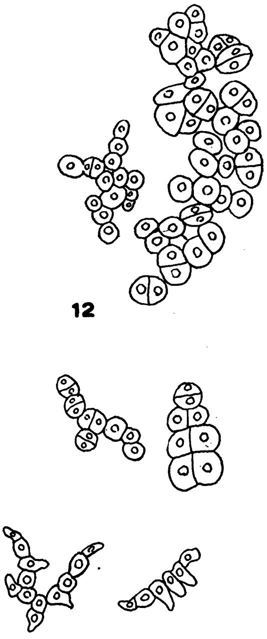
FIGURE 8. Palmella from *n*/10,000 $Co(NO_3)_2$.
FIGURE 9. Palmella and zoöspores from 5*n*/1,000,000 $CoSO_4$.
FIGURE 10. Filaments from *n*/1,000,000 $CoSO_4$.
FIGURE 11. Palmella from *n*/1,000,000 $Cu(NO_3)_2$.

(*Fig. 11* illustrates typical palmella from *n*/1,000,000 concentration.)

11. Copper sulfate ($CuSO_4$). — The sulfate again agrees with the nitrate. (*Fig. 12* shows the appearance of palmella in *n*/1,000,000, concentration.)

12. Hydrogen nitrate, nitric acid (HNO_3). — This body kills the plant at a concentration of 5*n*/10,000. Palmella is produced from *n*/10,000 to 6*n*/100,000, sometimes even in 3*n*/100,000. Acceleration of zoöspore production is exhibited from *n*/100,000 to 6*n*/100,000 or 3*n*/100,000, in certain cases, and below the last-named strength, at least at *n*/100,000, the alga appears normal. (See *fig. 13*, which shows palmella produced from filaments in a solution of 6*n*/100,000 concentration.)



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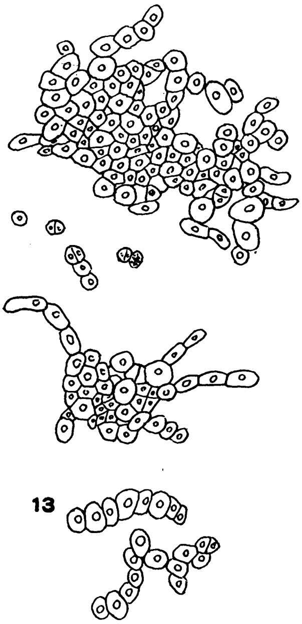

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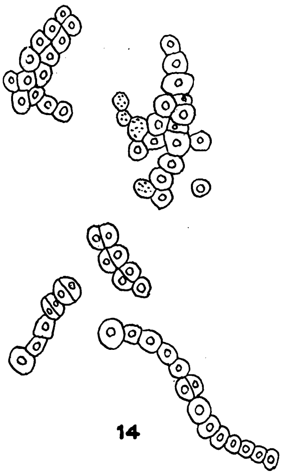
FIGURE 12. Palmella and some filaments changing to palmella, from *n*/1,000,000 $CuSO_4$.

FIGURE 13. Palmella from 6*n*/100,000 HNO_3.

13. Hydrogen sulfate, sulfuric acid (H_2SO_4). — This follows nitric acid very closely. There seems to be here sometimes a tendency for the palmella range to extend downward so to include *n*/100,000, but it was impossible to establish this for a certainty in all series. If it be so, it of course means that the sulfate is somewhat more active than the nitrate, and that the action in this case is not entirely to be attributed to the cation. This would agree with the results of certain other authors to be cited later. (*Fig. 14* shows palmella and dead cells from 3*n*/100,000.)

14. Iron nitrate (*ferric*) ($Fe_2(NO_3)_6$). — The cells are all killed at *n*/10,000. They live as the palmella form in concentrations of from 8*n*/100,000 to 5*n*/100,000. At 7*n*/100,000 and 8*n*/100,000 zoöspore production is accelerated, and at 2*n*/100,000 and *n*/100,000 the growth is normal for weak solutions. For these tests iron was omitted from the nutrient medium to which the poison was added.

15. Lead nitrate ($Pb(NO_3)_2$). — Death ensuses in *n*/10,000. In 5*n*/100,000, the majority of the cells die, but some become of the palmella form. The latter form is maintained through the series to 5*n*/1,000,000, in which solution there are usually a number of good filaments, although most of the culture is of spherical cells. Zoöspore acceleration occurs in *n*/100,000 to 5*n*/1,000,000. In *n*/1,000,000 to 5*n*/10,000,000, the growth of filaments and zoöspores is the same as in the control.



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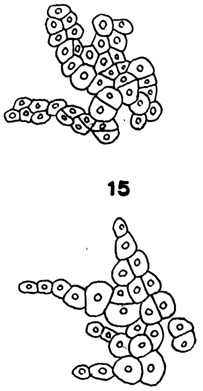

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FIGURE 14. Palmella and several dead cells from 3*n*/100,000 H_2SO_4.
FIGURE 15. Palmella from *n*/1,000,000 $AgNO_3$.

16. Lithium nitrate ($LiNO_3$). — Many, but not all, of the cells die in 5*n*/100. The palmella form is produced from 5*n*/100 to

5*n*/10,000, with some acceleration of zoöspore production in *n*/1,000 to 5*n*/10,000. The acceleration is not as marked as in most of the other salts, however. Normal growth occurs in *n*/10,000 and weaker solutions.

17. Lithium sulfate (Li_2SO_4). — A thorough test of this salt was not made, but as far as the experiments were carried the results agree well with the data given for the nitrate.

18. Magnesium nitrate ($Mg(NO_3)_2$). — This cation is present in the nutrient medium. The experiments consisted in increasing its amount. 5*n*/100 does not kill, but produces the palmella form as does also *n*/100 and, to some extent, 5*n*/1000. Zoöspore acceleration occurs in *n*/100 and the normal behavior is exhibited in *n*/1,000 and still weaker solutions.

19. Magnesium sulfate ($MgSO_4$). — This salt agrees with the nitrate perfectly excepting that it was impossible to establish a true zoöspore acceleration here. Sometimes this phenomenon appears and at other times not. It appeared most often at *n*/100 concentration. Perhaps undissociated molecules have to do with this response, but no particular study was made here, this subject lying rather in the field of nutrient salts than in that of toxic ions.

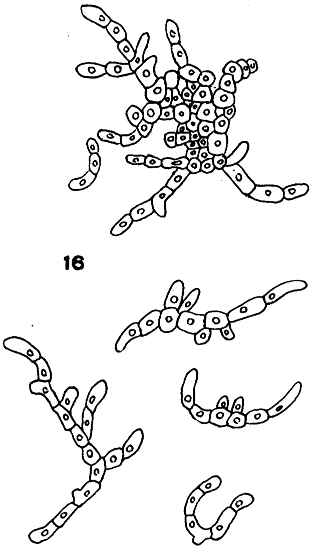
20. Nickel nitrate ($Ni(NO_3)_2$). — A concentration of *n*/10,000 produces death. Filaments are hardly at all present in 5*n*/100,000, practically all of the cells being of the other form, but in *n*/100,000 there is about an even mixture of the two forms. A rather slightly marked zoöspore acceleration occurs in *n*/100,000, and still less marked in weaker solutions. At a concentration of *n*/10,000,000 the alga is certainly uninfluenced by the poison. The zoöspore acceleration grades almost imperceptibly into the normal production of these bodies.

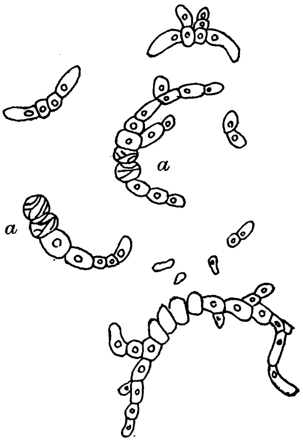
21. Potassium nitrate (KNO_3). — This salt is so common in the environment of plants that it would hardly be expected to produce very marked stimulation responses. It is probably less toxic toward plants in general than any other salt. At a concentration of 5*n*/100 the palmella form is produced. This is perhaps due, in part at least, to osmotic phenomena. No acceleration of zoöspore production was observed.

22. Potassium sulfate (K_2SO_4). — As far as could be determined, this salt acts exactly like the nitrate.

23. Rubidium sulfate (Rb_2SO_4). — The plant is killed by *n*/100 and to some extent by 5*n*/1,000. In 5*n*/1,000 and *n*/1,000 the palmella form is produced, with few or no zoospores, and the culture becomes normal at *n*/10,000. No acceleration of zoöspore production occurs here.

24. Silver nitrate ($AgNO_3$). — Death occurs from *n*/100,000 to 6*n*/1,000,000. Filaments do not appear in *n*/1,000,000, but begin to survive in 5*n*/10,000,000, where also there is an increase in the number of zoöspores. The cultures are normal or slightly accelerated as to zoöspore activity, in *n*/10,000,000. (See *figs. 15, 16,* and *17.* The former shows typical palmella form in *n*/1,000,000, the second a mixture of palmella and filamentous forms in 5*n*/10,000,000, and the last a normal filamentous culture in *n*/10,000,000. Zoösporangia are shown at *a.*) The limits are quite sharply defined for this salt.


16



17

FIGURE 16. Palmella and filaments from 5*n*/10,000,000 $AgNO_3$.
FIGURE 17. Normal filaments and zoösporangia (*a*), from *n*/10,000,000 $AgNO_3$.

25. Sodium nitrate ($NaNO_3$). — This cation is but little more poisonous than potassium. The palmella form is produced in

5*n*/100 to *n*/100, with filaments also quite well developed in the last-named strength. Osmotic pressure probably begins to play some part here. The killing concentration was not determined. There is little if any acceleration of zoöspore production. Cultures appear normal in *n*/1,000.

26. Sodium sulfate (Na_2SO_4). — This follows the last-named salt accurately as far as was determined.

27. Strontium nitrate ($Sr(NO_3)_2$). — This cation is very slightly toxic, so that here again osmotic phenomena begin to play a part before a stimulating concentration is reached. The killing strength was not determined. The palmella form is produced typically in 5*n*/100, and there is unquestionably an acceleration in zoöspore production in 5*n*/1,000, in which the zoöspores develop into short filaments but are destroyed rapidly by the formation of zoösporangia. The cultures are normal at *n*/1,000 and below.

28. Uranyl nitrate ($UO_2(NO_3)_2$). — Death occurs here in *n*/10,000 or stronger, the spherical form appears in 5*n*/100,000 and is found mixed with filaments in *n*/100,000. Acceleration of zoöspore production is exhibited in *n*/100,000 and in 5*n*/1,000,000, while normal growth occurs in *n*/1,000,000 and below.

29. Zinc nitrate ($Zn(NO_3)_2$). — In 5*n*/10,000 death ensues, in *n*/10,000 about one half of the cells die, the rest take the palmella form. In 5*n*/100,000 both filaments and palmella occur and zoöspores are more numerous than in the control, while in *n*/100 growth is the same as in the control.

30. Zinc sulfate ($ZnSO_4$). —The sulfate of this metal acts like the nitrate, excepting that here again an acceleration of zoöspore activity could not be established.

These results will now be brought together in tabular form. In the table which follows, the salts are arranged in the same order as in the foregoing description. In the first column after the name of the salt, occurs the lowest concentration producing death. In the second column are placed the concentrations at which filaments change to the other form. The strengths at which zoöspore production is accelerated are shown in column four, and that at which growth becomes normal is expressed in the last column.

TABLE OF RESULTS

Salt.	Lowest fatal concentration.	Filaments become palmella.	Acceleration of zoöspore production.	Normal filaments.
$Al_2(NO_3)_6$	n/10,000	5n/100,000 n/100,000	n/100,000 5n/1,000,000	n/1,000,000
$Al_2(SO_4)_3$	“	“	“	“
NH_4NO_3	?	n/100 5n/10,000	5n/10,000 6n/10,000	n/10,000
$(NH_4)_2SO_4$	?	“	“	“
$Ba(NO_3)_2$	n/100	5n/1,000 n/1,000	5n/1,000 n/1,000	5n/10,000
$Ca(NO_3)_2$	?	5n/100	n/100 5n/1,000	?
$Cd(NO_3)_2$	n/10,000	5n/100,000 n/100,000	n/100,000	5n/1,000,000
$Co(NO_3)_2$	n/1,000	5n/10,000 n/100,000	n/10,000 5n/1,000,000	n/1,000,000
$CoSO_4$	“	“	“	“
$Cu(NO_3)_2$	n/100,000	5n/1,000,000 5n/10,000,000	n/1,000,000	n/10,000.000
$CuSO_4$	“	“	“	“
HNO_3	5n/10,000	n/10,000 6n/100,000	n/100,000 6n/100,000	n/100,000
H_2SO_4	“	n/10,000 n/100,000 (?)	“	“
$Fe_2(NO_3)_6$	n/10,000	8n/100,000 5n/100,000	7n/100,000 8n/100,000	2n/100,000
$Pb(NO_3)_2$	n/10,000	5n/100,000 5n/1,000,000	n/100,000 5n/1,000,000	n/1,000,000
$LiNO_3$	?	5n/100 5n/10,000	n/1,000 5n/10,000 (slight)	n/10,000
Li_2SO_4	?	“	“	“
$Mg(NO_3)_2$	?	5n/100 n/100	n/100	n|1,000
$MgSO_4$	?	“	n/100 (?)	“
$Ni(NO_3)_2$	n/10,000	5n/100,000	n/100,000	n/10,000,000
KNO_3	?	5n/100	No acceleration.	?
K_2SO_4	?	“	No acceleration.	?
Rb_2SO_4	n/100	5n/1,000 n/1,000	No acceleration.	n/10,000
$AgNO_3$	n/100,000	6n/1,000,000 5n/10,000,000	5n/10,000,000	n/10,000,000
$NaNO_3$	?	5n/100 n/100	No acceleration.	n/1,000
Na_2SO_4	?	“	No acceleration.	“
$Sr(NO_3)_2$	?	5n/100	5n/1,000	n/1,000
$UO_2(NO_3)_2$	n/10,000	5n/100,000 n/100,000	n/100,000 5n/1,000,000	n/1,000,000
$Zn(NO_3)_2$	5n/10,000	n/10,000	5n/100,000	n/100,000
$ZnSO_4$	“	“	?	“

From the above statement of responses, it will readily be seen that the action of sulfate and nitrate is the same in practically all cases. This means, of course, that according to the dissociation hypothesis, the anions are comparatively without effect and that the responses just described are due to the presence of the metal ions in the medium. This is what should be expected from the fact that in the case of all the more poisonous salts the difference between experiment and control in concentration of the SO_4 or NO_3 ions is negligible. As has been said, these anions were chosen because they were already present in the nutrient medium. They are two out of the three which the plant uses most extensively in its metabolism, and thus it is not surprising that its protoplasmic system is of such a nature that slight, or even rather great, changes in their concentration are without visible effect upon the life-process. It will be remembered in this regard that it was shown in a previous paper * that a decrease to one-tenth its normal amount of any one of the nutrient salts used in Knop's solution is without effect upon this plant, so long as the osmotic properties of the solution are not altered by the change.

In order to study the question of the relation of stimulating power to the other properties of the metals studied, the following two lists were constructed. One is based on the lowest fatal concentrations, the other on the lowest concentration producing the palmella form. In the first column of each list is given the symbol of the cation (since anions play no part, they need not be considered), and in the second the concentration with regard to which the list is made. The elements are arranged in the order of their stimulating power, the weakest ones first. In cases where the least fatal concentration could not be determined with sharpness but where there are indications as to its position, slightly above or below the concentration given, the signs + and — are used to denote "greater than" and "less than." Where the sign is double it denotes " much greater than."

These lists are presented here in order to have the varying degrees of toxicity or stimulating power in mind before taking up the discussion of the three forms of response mentioned in a pre-

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* Livingston, B. E. On the nature of the stimulus which causes the change in form in polymorphic green algae. Bot. Gaz. 30 : 289-317. 1900.

ceding paragraph. These three responses will now be considered under the three heads: (1) The response of death, (2) The response in phenomena of growth, and (3) The response in reproduction.

List of cations according to lowest fatal concentration.		List of cations according to the lowest concentration producing palmella form.	
Element.	Concentration in Terms of *n*	Element.	Concentration in Terms of *n*
Ca	5/100 ++	Ca	5/100
Mg	5/100 ++	K	5/100
K	5/100 ++	Sr	5/100
Na	5/100 ++	Mg	1/100
Sr	5/100 ++	Na	1/100
Li	5/100 +	Ba	1/1,000
NH_4	1/100 +	Rb	1/1,000
Ba	1/100 +	NH_4	5/10,000
Rb	1/100 —	Li	5/10,000
Co	1/1,000	Zn	1/10,000
H	5/10,000	H	6/100,000
Zn	5/10,000 —	Fe	5/100,000
Fe	1/10,000	Ni	5/100,000
Ni	1/10,000	Al	1/100,000
U	1/10,000	Cd	1/100,000
Al	1/10,000 —	Co	1/100,000
Cd	1/10,000 —	U	1/100,000
Pb	1/10,000 —	Pb	5/1,000,000
Cu	1/100,000	Cu	5/10,000,000
Ag	1/100,000	Ag	5/10,000,000

III. DISCUSSION OF RESPONSES. 1. *The response of death.* — As has been stated, death must be considered as truly a response to stimuli as are any of the other alterations commonly passing under that name. Whatever may be the ultimate nature of the vital processes, they continue in such manner as to make up what is called life only while variation of the conditions external to the organism takes place within certain more or less narrow limits. Within these limits, changes of environment produce changes in the vital processes, and hence obvious changes in form, structure, and activity of the organism as a whole. Beyond them the necessary equilibrium of the many-sided system fails, and vitality ceases. Thus, the least concentration producing death may fairly be regarded as a criterion for estimating the stimulating power of any substance. The criterion for judging whether or not this plant is living is based on its loss of green color soon after death occurs.

No doubt all the salts tested here would produce death at *some* concentration, but those whose stimulating power or toxicity is weakest do not bring about this response until their concentration is high enough so that the osmotic pressure of the solution may begin to play its rôle. The cations Ca, Li, Mg, K, Na and Sr belong to this class, as do also probably NH_4, Ba, and perhaps Rb. The toxicity of these elements cannot be studied in a nutrient medium of as low pressure as the one here used. Perhaps it is impossible to determine it for the filamentous form of this plant.

What may be the nature of the killing power of the elements for which this property was determined is difficult to conjecture without more data. It is instructive to note, at any rate, that their effect in this regard is exactly similar to that of a drying medium. We see the same sort of death phenomena in these poisoned solutions of low osmotic pressure that was found for solutions of high pressure. In very strong solutions the cells die as filaments, in somewhat weaker ones they round off and assume the palmella form before death ensues. The relation of this toxicity to the physico-chemical nature of the elements will be considered in a later paragraph.

2. *The response in phenomena of growth.* — All of the elements tested produce the palmella response at some concentration. In case of Ca, K and Sr and perhaps of Mg and Na, this response may be in part due to osmotic pressure. For the other cations there can be no doubt that it is purely a chemical stimulus which is acting. The fact to be emphasized here again is that we have exactly the same growth changes brought about by the presence of toxic cations as are produced by physical extraction of water. In the poisoned cultures (whose osmotic pressure, it is to be remembered, is very low, much lower than is necessary for the retention of the filamentous form in an unpoisoned nutrient medium), there are observed exactly the same rounding up of cells, the same thickening of walls, and the same alteration in rapidity and direction of cell-division, as was found to take place when filaments are converted to the other form by the action of a concentrated solution.

These observations agree with those of the death responses, and it seems possible that in both cases we have to deal with a change in the water-content of the protoplasm. In the case of solutions of high osmotic pressure, water is extracted from the protoplasm directly, and it may be that this increasing density of the colloidal protoplasmic solution is accompanied by changes in its permeability to solute and in its general lability, and therefore chemical activity, which result in a higher osmotic pressure within the vacuole. I have been able to demonstrate this higher pressure by the plasmolytic method in cells of the palmella form. This higher osmotic pressure, as has been shown (*loc. cit.*, 1900), will suffice to explain the change in form of the cells and their partial or complete separation as they become spherical. A decrease in lability, and hence in general activity, may result in the observed thickening of the wall and in a change in the manner of cell-division, as is known to be the case in many plant and animal tissues under the influence of dryness and low temperature, where these are unquestionably concomitants of a decrease in the intensity of vital action.

But in case of toxic stimulation, with which this paper has to deal, there is no direct extraction or witholding of water from the cell, and by the principles of physics alone we are unable to see any difference between the poisoned and the unpoisoned nutrient medium. However, it is possible that the chemical stimulus of the toxic ions may be transformed, as the disturbance of the system passes within the limits of the protoplasm, and may become in this way a physical disturbance. It is well known that certain mineral salts hasten the coagulation of some proteids and other colloidal solutions,* and it is quite reasonable to suppose that the toxic ions upon entering the protoplasmic mass may produce in this vital hydrosol mixture an incipient coagulation or tendency toward the gel phase. If this were true it would mean that the colloidal particles become aggregated to some extent into denser masses, between and around which would lie a solution of less density, which would contain fewer colloidal particles than before. Thus, although by this supposed process of incipient coagulation water is not extracted from the protoplasmic solution as a whole, yet it is extracted from *certain parts* of this solution, namely from the partially coagulated groups or masses of colloidal particles. And if, as is probable, the seat of vital activity lies in the colloidal portion of the protoplasm rather than in the permeating aqueous solution, it becomes possible to see how an incipient production of the hydrogel phase in certain regions throughout the protoplasm may result in the same sort of alterations in permeability and lability as those brought about by general extraction of water with its accompanying increase in density of the vital substance. In brief, the suggestion here put forward is, that toxic ions may virtually bring about an extraction of water from the vital portions of the protoplasmic mass, and that this may result in the same obvious and tangible responses as those caused by direct extraction of water from the whole mass. Before this suggestion can be taken seriously it will be necessary to know more of the influence of ions upon colloidal solutions.

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* See Whetham, W. C. D. The coagulative power of electrolytes. Phil. Mag. V. 48 : 474–477. 1899.

Another possibility, closely related to the one just presented, is that the toxic cations may act upon the enzymes of the vital substance in such a manner as to bring about the changes noted. Indeed, the alteration of enzyme action (which appears in these days to have come to mean much the same as vital action, at least to play the leading part in our conception of the latter) may well be the common effect of water extraction and the entrance of poison cations into the living substance. Both suggestions are thus seen to be possible at the same time, the latter becoming a part of the former. The probability of the last idea, can be judged best only after more data have been obtained on the effect of poisons upon enzyme action, which work has happily already begun. *

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* See the following papers on this subject:

Zoethout, W. D. On the production of contact irritability without the precipitation of Ca salts. Am. Jour. Physiol. 10 : 324–334. 1904.— Further experiments on the influence of various electrolytes upon the tone of skeletal muscles. Am. Jour. Physiol. 10 : 373–377. 1904.

Brown, O. H. Effects of certain salts on kidney excretion with especial reference to glycosuria. Am. Jour. Physiol. 10 : 378–383. 1904.

Neilson, C. H. & Brown, O. H. Effect of ions on decomposition of hydrogen peroxide and hydrolysis of butyric ether by watery extracts of pancreas. Am. Jour. Physiol. 10 : 335–344. 1904.—Effects of ions on decomposition of hydrogen peroxide by platinum black. Am. Jour. Physiol. 10 : 225–228. 1904.

Neilson, C. H. The hydrolysis and synthesis of fats by platinum black. Am. Jour. Physiol. 10 : 191–200. 1903.

McGuigan, H. Relation between decomposition tension of salts and their antifermentative properties. Am. Jour. Physiol. 10 : 444–451. 1904.

3. The response in phenomena of reproduction. — With most of the cations studied there is a curious difference between the effect of drying solutions (of high pressure) and poisoned ones. This lies in the observed fact that in the latter zoöspore production may not only be as active as in the unpoisoned control, but that it may be much more active, while in the former zoöspore production is entirely inhibited. Thus extraction of water produces the palmella form and inhibits zoöspores, while the presence of toxic cations produces the palmella form, but also, at some concentration, generally accelerates the production of zoöspores. This would seem to prove that the relation between the two sets of responses is not as simple as might otherwise be supposed. It seems possible that the production of zoöspores is due to a somewhat different set of changes in the protoplasm from those involved in ordinary growth, a set of changes which may be started by several apparently different external factors. The phenomena of reproduction lie at present so much within the province of the unknown that it would probably be unprofitable to attempt any hypothesis in this regard. Attention may be called here, however, to the fact that there are fairly well-known activities taking place simultaneously within plant protoplasm, and yet controlled by entirely different sets of factors. As examples may be mentioned the processes of photosynthesis and respiration.

The fact that the acceleration here held in view is not observed in case of the cations, K, Rb, and Na, and is somewhat questionable in that of Li and Mg, together with the fact that these elements are among the least toxic, seems to show that there is some sort of a relation between the responses of death and change in growth, on the one hand, and of reproduction on the other. This matter of stimulation of reproductive activity may be a fertile field for further investigation.

Lillie, R. S. Relation of ions to ciliary movement. Am. Jour. Physiol. 10 : 419–443. 1904.

Mathews, A. P. The nature of chemical and electrical stimulation. I. The physiological action of ions depends on electrical state and electrical stability. Am. Jour. Physiol. 11 : 445–496. 1904.

Cole, S. W. Contributions to our knowledge of enzyme action. I. Influence of electrolytes on action of amylolytic ferments. Jour. Physiol. 30 : 202–220. 1903. — Contributions to our knowledge of enzyme action. II. Influence of electrolytes on the action of invertin. Jour. Physiol. 30 : 281–289. 1903.

LITERATURE

In any consideration of the general relation of the properties of reagents to those of responding organisms, it is essential to have a considerable mass of data from different forms of the latter. It is also desirable, of course, to know the relative stimulating or toxic power of as many elements as possible upon the same organism. The literature contains a respectable number of titles dealing with the question before us, but unfortunately the majority of the determinations have been made in such a manner as to render them quite inadequate for the present purpose. The work that has been done on plants has been briefly summarized, up to the time of his publication, by Copeland,* and a more recent review of certain phases of the subject comes from the hand of Benecke.† Observations which have an immediate relation to the results here presented will be stated briefly in the following paragraphs.

Acceleration of growth in *Aspergillus* and *Penicillium* was studied by Richards,‡ and in part corroborated by Miss Watterson. § For the former of these fungi $ZnSO_4$ accelerates growth most at concentrations of 0.002 per cent. (6*n*/100,000) to 0.004 per cent. (12*n*/100,000). The salt is harmful at concentrations from 0.05 per cent. (16*n*/10,000) to 0.075 per cent. (24*n*/1,000). $FeSO_4$ accelerates growth a little at 0.2 per cent. (26*n*/1,000), and the culture is normal at 0.033 per cent. (44*n*/10,000). $CoSO_4$ accelerates both fungi most at 0.002 per cent. (26*n*/100,000), while $NiSO_4$ shows the same response at 0.003 per cent. (85*n*/100,000). I have translated the percentage figures approximately into terms of a normal solution, and placed them in the parenthesis which follows each figure.

Ono || worked with both algae and fungi. His results may be

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* Copeland, E. B. Chemical stimulation and the evolution of carbon dioxid. Bot. Gaz. 35 : 81–98, 160–183. 1903.

† Benecke, W. Einige neuere Untersuchungen über den Einfluss von Mineralsalzen auf Organismen. Bot. Zeitung 62²: 113–126. 1904.

‡ Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. 30 : 665–679. 1897.

§ Watterson, A. The effect of chemical irritation on the respiration of fungi. Bull. Torrey Club 31 : 291–303. 1904.

|| Ono, N. Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize. Jour. Coll. Sci. Imp. Univ. Tokyo 13 : 141–186. 1900. Also Bot. Mag. Tokyo, 14 : 75. 1900.

tabulated as follows. Underneath his molecular concentrations I have placed the equivalent strength in terms of normal.

TABLE OF RESULTS OBTAINED BY ONO

Subject.	Salt.	Concentration for acceleration of vegetative growth.	Injurious concentration.	Normal growth.
Protococcus.	$ZnSO_4$	$\frac{1}{2}\ 10^{-6}m$ — $10^{-5}m$ $25n/100{,}000{,}000$ — $5n/1{,}000{,}000$	$\frac{1}{2}\ 10^{-4}m$ $25n/1{,}000{,}000$	
	$CoSO_4$	$\frac{1}{25}\ 10^{-5}m$ — $10^{-5}m$ $2n/1{,}000{,}000$ — $5n/1{,}000{,}000$	$\frac{1}{2}\ 10^{-4}m$ $25n/1{,}000{,}000$	
	$HgCl_2$	No acceleration.	$\frac{1}{25}\ 10^{-5}m$ $2n/10{,}000{,}000$	
	NaF	$\frac{1}{125}\ 10^{-3}m$ — $\frac{1}{5}\ 10^{-3}m$ $8n/1{,}000{,}000$ — $4n/10{,}000$	$10^{-3}m$ $n/1{,}000$	
	$LiNO_3$	$\frac{1}{25}\ 10^{-4}m$ — $10^{-4}m$ $4n/1{,}000{,}000$ — $n/10{,}000$	$\frac{1}{2}\ 10^{-3}m$ $5n/10{,}000$	
	K_3AsO_4	$\frac{1}{125}\ 10^{-4}m$ — $\frac{1}{25}\ 10^{-4}m$ $26n/100{,}000{,}000$ — $13n/10{,}000{,}000$	$10^{-4}m$ $33n/1{,}000{,}000$	$\frac{1}{5}\ 10^{-4}m$ $66n/10{,}000{,}000$
Chroococcum.	$ZnSO_4$	$\frac{1}{25}\ 10^{-5}m$ — $10^{-5}m$ $2n/10{,}000{,}000$ — $5n/1{,}000{,}000$		$\frac{1}{2}\ 10^{-4}m$ $25n/1{,}000{,}000$
	$CuSO_4$	No acceleration.	$\frac{1}{5}\ 10^{-5}m$ $n/1{,}000{,}000$	$\frac{1}{25}\ 10^{-5}m$ $2n/10{,}000{,}000$
Hormidium.	$FeSO_4$	$\frac{1}{25}\ 10^{-4}m$ — $\frac{1}{2}\ 10^{-3}m$ $2n/1{,}000{,}000$ — $25n/100{,}000$		
	$NiSO_4$	$\frac{1}{25}\ 10^{-5}m$ — $\frac{1}{5}\ 10^{-4}m$ $2n/10{,}000{,}000$ — $n/100{,}000$	$\frac{1}{2}\ 10^{-4}m$ $25n/1{,}000{,}000$	$10^{-5}m$ $5n/1{,}000{,}000$
	$CoSO_4$	$\frac{1}{25}\ 10^{-5}m$ $2n/10{,}000{,}000$	$\frac{1}{5}\ 10^{-5}m$ $n/1{,}000{,}000$	$\frac{1}{2}\ 10^{-4}m$ $25n/1{,}000{,}000$
Stigeoclonium.	$CuSO_4$	No acceleration.	$\frac{1}{5}\ 10^{-5}m$ — $\frac{1}{25}\ 10^{-5}m$ $n/1{,}000{,}000$ — $2n/10{,}000{,}000$	
Aspergillus.	$ZnSO_4$	$\frac{1}{8}\ 10^{-3}m$ — $10^{-3}m$ $6n/100{,}000$ — $5n/10{,}000$		
	$NiSO_4$	$\frac{1}{8}\ 10^{-3}m$ — $10^{-3}m$ $6n/100{,}000$ — $5n/10{,}000$		
	$CoSO_4$	$\frac{1}{16}\ 10^{-3}m$ — $\frac{1}{2}\ 10^{-3}m$ $3n/100{,}000$ — $25n/100{,}000$		
	$CuSO_4$	$\frac{1}{16}\ 10^{-3}m$ — $\frac{1}{2}\ 10^{-3}m$ $3n/100{,}000$ — $25n/100{,}000$		
	$HgCl_2$	$\frac{1}{8}\ 10^{-4}m$ — $\frac{1}{16}\ 10^{-3}m$ $6n/1{,}000{,}000$ — $3n/100{,}000$	$\frac{1}{8}\ 10^{-3}m$ $3n/100{,}000$	
	$LiNO_3$	$\frac{1}{16}\ 10^{-2}m$ — $\frac{1}{2}\ 10^{-2}m$ $6n/10{,}000$ — $5n/100$		
	NaF	$\frac{1}{16}\ 10^{-2}m$ — $\frac{1}{8}\ 10^{-2}m$ $6n/10{,}000$ — $125n/100{,}000$	$\frac{1}{2}\ 10^{-2}m$ $5n/100$	$\frac{1}{4}\ 10^{-2}m$ $25n/10{,}000$
Penicillium.	$FeSO_4$	$\frac{1}{4}\ 10^{-3}m$ — $\frac{1}{2}\ 10^{-3}m$ $125n/1{,}000{,}000$ — $25n/100{,}000$	$2 \times 10^{-3}m$ $n/1{,}000$	$10^{-3}m$ $5n/100$
	$CoSO_4$	$\frac{1}{16}\ 10^{-3}m$ — $\frac{1}{2}\ 10^{-3}m$ $3n/100{,}000$ — $25n/100{,}000$		
	$HgCl_2$	$\frac{1}{16}\ 10^{-3}m$ — $\frac{1}{2}\ 10^{-3}m$ $3n/100{,}000$ — $25n/100{,}000$		

This author observed no acceleration in reproductive activity. The accelerations which he noted, — and they occurred for algae with all salts excepting $AgCl_2$ and $CuSO_4$, and with all salts tried for fungi,— always consisted in a hastening of the growth rate, thus giving weight of yield greater than that of the control. His form of *Stigeoclonium* was different from mine. Nothing is said about its being polymorphic, while with my form the important responses are all those which are bound up in the physiology of polymorphism. This difference in the response of acceleration would seem to indicate that even the most nearly related organisms may react differently towards the same stimuli. The concentration limits were not determined sharply enough to make any rigid comparison of these profitable. As far as comparison can be made the results of my experiments seem to agree in a general way with those obtained by Ono.

Richter * was unable to get an acceleration of growth in *Aspergillus* with Cu although he obtained this response with Zn. It may be that in these lower forms there are great physiological variations within a single species. This would account for some of the apparent discrepancy in regard to the effects of these salts which have been tried so often. For a description of the behavior of a form of *Penicillium* peculiarly resistant to copper and one extraordinarily resistant to arsenic, see the work of De Seynes † and Gosio. ‡

Stevens § tested the influence of a number of salts in inhibiting the germination of spores of several fungi. The following table compiled from one of his, gives the lowest inhibiting concentrations for the forms there named, with respect to those salts which he and I have both tested. I have corrected his terminology from *n* to *m*, and have added underneath his figures the approximately equivalent concentration in the normal and decimal system. The sign > preceding a concentration denotes that the limit as stated is somewhat too low.

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* Richter, A. Zur Frage der chemischen Reizmittel. Centralbl. für Bacteriol. **7**: 417–429. 1901.

† De Seynes, J. Resultats de la culture du *Penicillium cupricum* Trabut. Bull. Soc. Bot. France **42**: 451–455, 482–485. 1895.

‡ Gosio, B. Zur Frage wodurch die Giftigkeit arsenhaltiger Tapeten bedingt wird. Ber. Deutsch. Chem. Ges. **30**: 1024–1026. 1897.

§ Stevens, F. L. The effect of aqueous solutions upon the germination of fungus spores. Bot. Gaz. **26**: 377–406. 1898.

TABLE OF CERTAIN RESULTS OBTAINED BY STEVENS: LOWEST INHIBITING CONCENTRATION

Salt.	*Botrytis.*	*Macrosporium.*	*Gloeosporium.*	*Penicillium.*	*Uromyces.*
HCl	....	$> m/50$ $> 2n/100$	$> m/100$ $> n/100$	$> m/50$ $> 2n/100$	$m/50$ $2n/100$
H_2SO_4	....	$> m/50$ $> n/100$	$> m/100$ $> 5n/1,000$	$> m/50$ $> n/100$	$m/50$ $> n/100$
$CuSO_4$	$m/3,200$ $15n/10,000$	$m/6,400$ $75n/100,000$	....	$m/200$ $25n/10,000$	$m/3,200$ $15n/10,000$
$Cu(NO_3)_2$	$m/3,200$ $15n/10,000$	$> m/3,200$ $> 15n/10,000$	....	$m/200$ $25n/10,000$	$m/3,200$ $15n/10,000$
NH_4NO_3	$m/2$ $5n/10$	....	....	$m/2$ $5n/10$	$m/2$ $5n/10$
$MgSO_4$	....	....	....	$> m/10$ $> 5n/100$	....

Clark * worked in a field similar to that of Stevens, making valuable determinations of the effect of poisons upon fungus growth as well as upon the germination of spores. Below are presented those of his results which are of most interest in the present connection. The figures denote the lowest fatal strength for the organisms indicated. I have added the decimal equivalent as above, placing it to the right of the sign =, and have arranged the salts in the order of their toxicity.

TABLE OF CERTAIN RESULTS OBTAINED BY CLARK

Aspergillus.		*Sterigmatocystis.*	
Salt.	Fatal concentration.	Salt.	Fatal concentration.
$CoSO_4$	$n = n$	$CoSO_4$	$n = n$
HCl	$n/4 = 25n/100$	$ZnSO_4$	$n = n$
$CuSO_4$	$n/4 = 25n/100$	HCl	$n/2 = 5n/10$
$Cu(NO_3)_2$	$n/4 = 25n/100$	HNO_3	$n/4 = 25n/100$
H_2SO_4	$n/8 = 125n/1,000$	$Ni(NO_3)_2$	$n/4 = 25n/100$
HNO_3	$n/16 = 625n/10,000$	$Cu(NO_3)_2$	$n/4 = 25n/100$
$Cd(NO_3)_2$	$n/64 = 156n/10,000$	$CuSO_4$	$n/8 = 125n/1,000$
$AgNO_3$	$n/2,048 = 49n/100,000$	H_2SO_4	$n/16 = 625n/10,000$
		$Cd(NO_3)_2$	$n/32 = 313n/10,000$
		$AgNO_3$	$3n/4,096 = 245/1,000,000$
Penicillium.		*Penicillium.*	
Salt.	Fatal concentration.	Salt.	Fatal concentration.
$CoSO_4$	$n = n$	H_2SO_4	$n/2 = 5n/10$
$CuSO_4$	$n = n$	$Ni(NO_3)_2$	$n/2 = 5n/10$
$Cu(NO_3)_2$	$n = n$	HNO_3	$n/4 = 25n/100$
$ZnSO_4$	$n = n$	$Cd(NO_3)_2$	$n/256 = 39n/10\ 000$
HCl	$n/2 = 5n/10$	$AgNO_3$	$n/32,768 = 305n/10,000,000$

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* Clark, J. F. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**: 289–327, 378–404. 1899. — Electrolytic dissociation and toxic effect. Jour. Phys. Chem. **3**: 263–316. 1899.

Oedocephalum.		*Botrytis.*	
Salt.	Fatal concentration.	Salt.	Fatal concentration.
$CoSO_4$	$2n = 2n$	$ZnSO_4$	$n = n$
$ZnSO_4$	$n/2 = 5n/10$	$CoSO_4$	$n/4 = 25n/100$
HCl	$n/8 = 125n/1,000$	HCl	$n/8 = 125n/1,000$
HNO_3	$n/8 = 125n/1,000$	HNO_3	$n/16 = 625n/10,000$
H_2SO_4	$n/16 = 625n/10,000$	$CuSO_4$	$n/32 = 313n/10,000$
$Ni(NO_3)_2$	$n/16 = 625n/10,000$	$Cu(NO_3)_2$	$n/32 = 313n/10,000$
$CuSO_4$	$n/64 = 156n/10,000$	H_2SO_4	$n/64 = 156n/10,000$
$Cu(NO_3)_2$	$n/64 = 156n/10,000$	$Ni(NO_3)_2$	$n/128 = 78n/10,000$
$Cd(NO_3)_2$	$n/128 = 78n/10,000$	$Cd(NO_3)_2$	$n/4,096 = 245n/1,000,000$
$AgNO_3$	$n/32,768 = 305n/10,000,000$	$AgNO_3$	$n/8,192 = 123n/1,000,000$

The results of these studies of the relative toxicity of the metals toward algae and fungi are presented below in the form of lists, so that they may be more readily compared. The salts studied are arranged in order of their stimulating effect — beginning with the least effective — under the name of the subject. A vertical line at the left of several salts denotes their apparent equality in the series. Superscript letters attached to the subject names denote authors, as follows : (*a*) Richards ; (*b*) Ono ; (*c*) Livingston ; (*d*) Clark ; and (*e*) Stevens.

STIMULATION.

Aspergillus[a].	*Aspergillus*[b].	*Penicillium*[b].	*Protococcus*[b].	*Stigeoclonium.*[c]
$FeSO_4$	$HgCl_2$	$FeSO_4$	$ZnSO_4$	Ca
$NiSO_4$	$CuSO_4$	$CoSO_4$	$CoSO_4$	K
$CoSO_4$	$CoSO_4$	$HgCl_2$	$LiNO_3$	Sr
$ZnSO_4$	$NiSO_4$			Mg
	$ZnSO_4$			Na
	$LiNO_3$			Ba
				Rb
				NH_4
				Li
				Zn
				H
				Fe
				Ni
				Al
				Cd
				Co
				U
				Pb
				Cu
				Ag

FATAL STRENGTH

Aspergillus[d].	*Penicillium*[e].	*Penicillium*[d].	*Protococcus*[b].	*Botrytis*[e].	*Botrytis*[d].
$CoSO_4$	NH_4NO_3	$CoSO_4$	$HgCl_2$	NH_4NO_3	$ZnSO_4$
HCl	$MgSO_4$	$CuSO_4$	$ZnSO_4$	$CuSO_4$	$CoSO_4$
$CuSO_4$	HCl	$Cu(NO_3)_2$	$CoSO_4$	$Cu(NO_3)_2$	HCl
$CuNO_3$	H_2SO_4	$ZnSO_4$	$LiNO_3$		HNO_3
H_2SO_4	$CuSO_4$	HCl			$CuSO_4$
HNO_3	$Cu(NO_3)_2$	H_2SO_4			$CuNO_3$
$Cd(NO_3)_2$		$Ni(NO_3)_2$			H_2SO_4
$AgNO_3$		HNO_3			$Ni(NO_3)_2$
		$Cd(NO_3)_2$			$Cd(NO_3)_2$
		$AgNO_3$			$AgNO_3$

Macrosporium[e].	*Uromyces*[e].	*Sterigmatocystis*[d].	*Oedocephalum*[d].	*Stigeoclonium*[c].
HCl	NH_4NO_3	$CoSO_4$	$CoSO_4$	Ca
H_2SO_4	HCl	$ZnSO_4$	$ZnSO_4$	Mg
$Cu(NO_3)_2$	H_2SO_4	HCl	HCl	K
$CuSO_4$	$CuSO_4$	HNO_3	HNO_3	Na
	$Cu(NO_3)_2$	$Ni(NO_3)_2$	H_2SO_4	Sr
		$Cu(NO_3)_2$	$Ni(NO_3)_2$	Li
		$CuSO_4$	$CuSO_4$	NH_4
		H_2SO_4	$Cu(NO_3)_2$	Ba
		$Cd(NO_3)_2$	$Cd(NO_3)_2$	Rb
		$AgNO_3$	$AgNO_3$	Co
				H
				Zn
				Fe
				Ni
				U
				Al
				Cd
				Pb
				Cu
				Ag

A comparison of the above lists will make it clear at once that there is only a very general agreement between them. In the present state of our knowledge nothing more need be said.

The literature of the effect of toxic substances on higher plants, although more extensive, is in a more unsatisfactory state than that concerning the lower ones. It would be somewhat out of the field of the present paper to go here into detail as to the concentrations which accelerate growth and those which kill higher plants. It is well established that most of the metals, when at the right strength, do accelerate growth of seedling roots, but there is discrepancy among the several observers as to the limits. The same is true of fatal doses. Kahlenberg and True * and Heald[*] worked upon *Lupinus*, *Pisum* and *Zea* radicles, finding an acceleration in case of the cations H, Cu, Ni, Co and Ag. Kahlenberg and Austin[†] determined the concentration of ionic H which can be borne by seedlings of *Lupinus* when their roots are in solutions of acid sodium salts. Coupin[‡] made determinations of the effect of solutions of salts of Na, K, and NH_4 upon wheat seedlings. Kanda[§] worked with *Pisum* and *Vicia* seedlings and with $CuSO_4$ and $ZnSO_4$. Studies on the toxicity of a number of acids and their Ca and K salts upon wheat, clover, and maize, were made by Cameron and Breazeale.[||] True and Gies[¶] have recently carried out what is probably now the most satisfactory research we have on this subject. They determined for *Lupinus* the stimulating strengths and fatal concentrations for the salts of Cu, Ag, Hg, Zn, Na, K, Ca and Mg. F. A. Loew[**] worked upon the effect of H and OH ions on *Zea* seedlings, and Dandeno[††] has just published a paper on experiments in this line with seedlings of *Zea*, *Lupinus*, and *Pisum*. The latter author made determinations of the neutralizing power of seedling roots in toxic solutions, which appears to be a very important consideration and one which seems not to have been attacked before. This author demonstrates also, what had already been described by True and Oglevee[‡‡] that the presence of insoluble bodies has an influence upon the physiological effect of salt solutions. This is the phenomenon of adsorption known to soil physicists for some time,* but only now applied to the study of toxicity of salts. It appears from Dandeno's paper that even the walls of the vessel in which a water culture is grown may exert an appreciable influence in this way, thus decreasing the stimulating power of a solution.

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* Kahlenberg, L. & True, R. H. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. 22 : 81–124. 1896.

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[*] Heald, F. de F. On the toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz. **22**: 125–153. 1896.

[†] Kahlenberg, L. & Austin, R. W. The action of acid sodium salts on *Lupinus albus*. Jour. Phys. Chem. **4**: 553–569. 1900.

[‡] Coupin, H. Sur la toxicité des composés du sodium, du potassium, et de l'ammonium à l'égard des végétaux supérieurs. Rev. Gén. Bot. **12**: 177–194. 1900.

[§] Kanda, M. Studien über die Reizwirkung einiger Metallsalze auf das Wachsthum höherer Pflanzen. Jour. Coll. Sci. Imp. Univ. Tokyo 19^{13}: 1–37. 1904.

[||] Cameron, F. K. & Breazeale, J. F. The toxic action of acids and salts on seedlings. Jour. Phys. Chem. **8**: 1–13. 1904.

[¶] True, R. H. & Gies, W. J. On the physiological action of some of the heavy metals in mixed solutions. Bull. Torrey Club **30**: 390–402. 1903.

[**] Loew, F. A. The toxic effect of H and OH ions on seedlings of Indian corn. Science II. **18**: 304–308. 1903.

[††] Dandeno, J. B. The relation of mass action and physical affinity to toxicity. Am. Jour. Sci. IV. **17**: 437–458. 1904.

[‡‡] True, R. H. & Oglevee, C. S. The effect of the presence of insoluble substances on the toxic action of poisons. Science II. **19**: 421–424. 1904. — Also Bot. Gaz. **39**: 1–21. 1905.

It is well to call attention here also to three papers in the same field in animal physiology. The influence of poison salts upon fishes was studied by Kahlenberg and Mehl † and determination made of the killing concentrations. As their results show, these authors were working largely with the toxic effect of the solutions upon the delicate vascular membranes of the gills. Kahlenberg ‡ also made a study of the relation of taste to acidity, in salts and acids. But by far the best and most satisfactory paper which has appeared from the animal side, and in many ways from the standpoint of all general physiology, is that of Mathews § who carefully determined for a large number of salts, the concentrations necessary to inhibit the development of eggs of the fish, *Fundulus heteroclitus*. His paper will be discussed to some degree a little farther on.

The effect of one salt or ion in counteracting that of another, when these are in a mixed solution is a very important topic in connection with the general subject of stimulation, Krönig and Paul,|| Clark,¶ Kearney and Cameron,** True,†† and True and Gies,§§ and others have investigated this question with interesting result which cannot be even touched upon here.

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* See Briggs, L. J. The mechanics of soil moisture. U. S. Dept. Agric. Div. of Soils, Bull. No. 10. 1897. — Investigations on the properties of soils. U. S. Dept. Agric. Field Operations Div. Soils, 1900 : 415–421. 1901.

† Kahlenberg, L. & Mehl, H. F. Toxic action of electrolytes upon fishes. Jour. Phys. Chem. 5 : 113–132. 1901.

‡ Kahlenberg, L. The relation of the taste of acid salts to their degree of dissociation. Jour. Phys. Chem. 4 : 533–537. 1900.

§ Mathews, A. P. The relation between solution tension, atomic volume, and the physiological action of the elements. Am. Jour. Physiol. 10 : 290–323. 1904. — See also his previous paper on nerve irritability, Science II. 17 : 728–733. 1903.

|| Krönig, B. & Paul, T. Zeitschr. für Hygiene u. Infect. 25 : 1–112. 1897.

¶ Clark, J. F. Jour. Phys. Chem. 5 : 289–316. 1901.

** Kearney, T. H. and Cameron, F. K. U. S. Dept. Agric. Report No. 71. 1902.

†† True, R. H. Am. Jour. Sci. IV. 9 : 183–192. 1900.

§§ True, R. H. & Gies, W. J. Bull. Torrey Club 30 : 390–402. 1903.

NATURE OF TOXICITY

Although a number of attempts have been made to find out what it is in the nature of these toxic substances which gives them their stimulating power, there has been practically no outcome of it all until the appearance of Mathew's paper above referred to. Practically all of the toxicity series which have been made out seem to agree in certain respects, *e. g.*, they do not follow the valence of the elements involved, nor do they follow the order of atomic weights; on the other hand, it is usually evident that, in some sort of a general way, they do depend upon the chemical nature of these elements, the more inert atoms always appearing as of low stimulating power while Cu, Ag, Pt, etc., lie at the opposite end of the series. This much is as evident from my work as it is from that of Kahlenberg and True, Clark and Mathews.

The last-named author has discovered a remarkable similarity between his toxicity series and three chemical series formed on the basis, respectively, of *solution tension*, *atomic volume*, and a *function obtained by dividing the equivalent weight by the atomic volume*. With none of these series my results agree accurately, but a comparison of his published tables with those here presented (page 20) will show some remarkable points of resemblance. It would appear from a comparison of all the work available on this subject that, while the suggestion of the author just named seems to fall short of explaining the relation of toxicity in general to chemical properties, yet he has at least given us the only rational basis for exploration of this difficult field.

That different organisms behave differently in the same solution is to be expected from the mere fact that they *are* different organisms, *i. e.*, that their protoplasms are not identical. The points of similarity in different protoplasms have become so strongly emphasized (as is illustrated by the fact that we use the single term protoplasm to include them all), that their essential points of difference have often been partially lost sight of. The toxicity series for any form is doubtless conditioned by a complex function derived, on the one hand, from the properties of its protoplasm as an organic mixture and a colloidal solution, and, on the other, from some such properties of the elements as those considered by Mathews.

It would be unnecessarily increasing the bulk of literature to attempt a discussion of the discrepancies between the different series at hand. It is almost certain that the present lively activity of physiologists in this regard will soon unearth additions to our knowledge of the facts which will be vastly more valuable than any amount of the older-fashioned *a priori* discussion of all the conceivable possibilities of the case. The writer hopes in the near future to study in a similar manner the influence of anions and of certain organic poisons upon this organism.

SUMMARY

The important results here described may be briefly stated as follows :

1. Nitrate and sulfate, in the case of a large number of metallic elements, act in the same way and at the same concentration upon the filamentous form of this alga. According to the theory of dissociation, we conclude that the stimulation is due to the cations.
2. At high enough concentrations death is produced.
3. At somewhat lower concentrations most of the cations produce a change in form of the cells and in the manner of cell division, which is strictly parallel to the change brought about by extraction of water or inhibition of its absorption.
4. Often at the same concentration as that mentioned in (3), and in most cases at a strength somewhat lower than this, there is a marked acceleration in the production of zoöspores. This is exactly the opposite of what results from water-extraction.
5. The acceleration in zoöspore activity gradually decreases with weaker solutions of the poison until the normal behavior of the filamentous form is reached.
6. In general, the relative degrees of toxicity of the metals here studied follow the order of those studied by other workers with different organisms. But there are many unexplained discrepancies.

THE DESERT BOTANICAL LABORATORY OF THE
CARNEGIE INSTITUTION, TUCSON, ARIZONA, Aug. 5, 1904.